The effects of boron administration on plasma leptin and lactate levels in ovariectomized rats which had acute swimming exercise

Zubeyde Yazıcı¹, Yalcin Kaya¹, Abdulkerim Kasim Baltaci²*, Rasim Mogulkoc² and Esma Oztekin³

1. Selcuk University, Department of Physical Education and Sports Sciences, Konya-Turkey
2. Selcuk University, Meram Medical School, Department of Physiology, Konya-Turkey
3. Selcuk University, Meram Medical School, Department of Biochemistry, Konya-Turkey

Correspondence to: Dr. Abdulkerim Kasim Baltaci
Selcuk University, Meram Medical School, Department of Physiology
42080, Konya, Turkey
E-MAIL: baltaci61@yahoo.com

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Abstract

OBJECTIVES: The aim of this study was to investigate the effects of intraperitoneal (IP) boron administration on leptin and lactate levels in swimming exercise in ovariectomized rats.

METHODS: Eighty adult female rats were equally allocated into 8 groups. Group 1; Control, Group 2; exercise control, Group 3; ovariectomized control, Group 4; Boron control (2 mg/kg/day), Group 5; ovariectomized plus exercise, Group 6; exercise plus IP boron, Group 7; ovariectomized and exercise group plus IP boron, Group 8; ovariectomized plus boron.

RESULTS: Leptin levels in Group 1 were higher than those in Groups 2, 3 and 4 and lower than those in groups 5, 6, 7 and 8 (p<0.01). Leptin levels were higher in Group 3 than in Groups 2 and 4 and significantly lower in all other groups (p<0.01). Lactate levels in Groups 2 and 4 were higher than those in all other groups (p<0.01). Lactate levels were significantly lower in Groups 1, 3, 5 and 8 than in all other groups (p<0.01). Lactate levels in Groups 6 and 7 were significantly lower than those in Groups 2 and 4 (p<0.01) and higher than those in groups 1, 3 and 5 (p<0.01).

CONCLUSION: The present findings demonstrate that ovariectomy and acute swimming exercise in rats led to a significant decrease in leptin levels and a significant increase in lactate levels and that boron administration prevented the increased in circulating lactate concentration induced by swimming exercise.

INTRODUCTION

Recently there has been an increase in the claims that boron element, which is widely used in metallurgy, is also important for human health (Naghi & Samman, 1993). In fact, boron is present in various areas of human life from food to some personal care products. Although it has been known since 1857 that boron element is present in plants, research into its effects in animal and human metabolism is more recent (Naghi & Samman, 1993).
Studies carried out to discover the functions of boron in the body show that it contributes to a number of vital functions like mineral and energy metabolism, brain functions, hormonal balance and bone growth and although its biochemical functions have not been exactly identified yet. It is recommended to take food rich in boron such as vegetables, fruits, broad bean family and dried fruits because of their benefits for the body (Nielsen, 1997).

Amateur and professional athletes alike use several ergogenic supplements that are claimed to increase sporting performance. In several branches of sports, many athletes add ergogenic supplements to their diets, while others make sure that basic elements are included in their diets (Ahrent, 2001). One of the elements that is investigated in terms of its relation to sporting performance is boron. It is reported that dietary boron supplementation increases endogenic steroid production and side effects are seen only in high doses (Ahrent, 2001).

It is propounded that boron supplementation leads to an increase in testosterone levels and that dietary boron supplement may be beneficial for performance in individuals doing endurance exercise (Naghii, 1999). As it is reported, boron supplementation can be an ergogenic substance for athletes, since it increases steroid hormones and it is noted that the relation between boron and exercise should be investigated further (Naghii, 1999). However, no study was found in our literature search about boron-exercise-menopause relation.

The present study investigates not only the effects of boron administration on performance in ovariectomized and exercising female rats, but also the relation between boron administration and plasma leptin and lactate levels.

MATERIALS AND METHODS

Rats
Animal Materials and Groups: This study was carried out at Selcuk University (S.U), Experimental Medicine Research and Application Center (SUDAM) on 80 adult female rats weighing 200–250 g. of Spraque –Dawley species obtained from the same Center. SUDAM ethics committee approved the study protocol.

Experimental Design
Experimental animals were allocated to 8 groups as follows:
Group 1 (n=10) General Control Group: The group which was fed on a normal diet and which was not exposed to any procedure.
Group 2 (n=10) Exercise Control Group: The group that was fed on a normal diet for 6 weeks and made to do acute swimming exercise for 30 minutes at the end of the study period.
Group 3 (n=10) Ovariectomy Control Group: The group that was fed on a normal diet for 6 weeks three days after ovariectomy.
Group 4 (n=10) Boron Control Group: The group that was administered 2 mg/kg/day intraperitoneal boric acid per rat for 6 weeks.
Group 5 (n=10) Ovariectomy + Exercise Group: The group that was fed on a normal diet for 6 weeks after ovariectomy and that was made to do acute swimming exercise for 30 minutes at the end of the study period.
Group 6 (n=10) Boron + Exercise Group: The group that was administered 2 mg/kg/day intraperitoneal boric acid per rat for 6 weeks and that was made to do acute swimming exercise for 30 minutes at the end of the study.
Group 7 (n=10) Ovariectomy + Boron + Exercise Group: The group that was administered 2 mg/kg/day intraperitoneal boric acid per rat for 6 weeks after ovariectomy and that was made to do acute swimming exercise for 30 minutes at the end of the study.
Group 8 (n=10): Ovariectomy + Boron Group: The group which was ovariectomy and boron supplemented 2 mg/kg/day for 6 weeks.

Ovariectomy Procedure
Ovariectomy was performed under general anesthesia (60 mg/kg ketamin and 5 mg/kg rompun). Ovariectomy procedure was as follows: After the hair on the back of the rats was shaved, appropriate asepsis and antisepsis was ensured by betadin. Following that, rats were placed in ventral position and the skin was incised from 1/3 upper point of the stretch between medial part of the back and tail. Spinal muscle was reached after releasing subcutaneous tissues. Peritoneal cavity was accessed via the muscles on the back wall of the abdomen. Ovaries were taken out together with lipid tissue. Ovaries were cleared of the lipid tissue and clamped, ligated and cut. After hemorrhage was checked, other organs were put into the peritoneal cavity. The muscle was sutured with 2/0 chrome catgut and the skin with 2/0 silk (Waynfort & Flecnel, 1994).

Boric Acid Administration
Boron administration was prepared so as to have 2 mg of boron in 0.5 ml serum physiologic. 2mg/0.5ml serum physiologic: 22.87 H₃BO₃ was put into a one-liter volumetric flask and complemented to 1 liter with serum physiologic. Intraperitoneal boron administration to relevant rats was made daily at 12.00 a.m. for 6 weeks.

Swimming Exercise
Swimming exercise was done in a 50 x 50 cm heat-resistant glass pool with a thermostat to fixate the temperature at 37°C. Exercises were done once for 30 minutes at the end of the injection period. Experimental animals were made to swim in pairs. After swimming exercise was completed, they were decapitated and blood samples were collected for analysis (Kaya et al. 2006).
Biochemical Analysis Lactate Determinations
2 ml of blood samples put into fluoride-oxalate anticoagulant containing tubes were placed in ice blocks and within the short period of 15 minutes they were centrifuged at +4°C for five minutes at 3000 rotations to separate plasma. The separated plasma samples were studied in S.U. School of Medicine Central Biochemistry Laboratory with TECHNICON RA-XT brand autoanalyzer equipment using colorimetric method (Sigma Diagnostic lactic acid kits were used). Plasma lactate levels (read at 550 nm wavelength) were determined as mg/dl.

Leptin Determinations
Plasma leptin analysis was carried out using Rat Leptin RIA test kit (Linco trademark catalogue no: RL-83K). The results were presented as ng/ml. Limit sensitivity and limit linearity of the leptin analysis are 0.5ng/ml and 50ng/ml respectively.

Statistical Analysis
SPSS package software was used for statistical analysis. Kruskal-Wallis variance analysis and Mann-Whitney U test were carried out. Level of significance was set at p<0.05. Values of leptin and lactate in study groups were presented as Mean ± SD.

RESULTS
Plasma leptin levels were summarized in Figure 1. Plasma leptin levels of Groups 5, 6, 7 and 8 were higher than those in all other groups (p<0.01, Figure 1). Plasma leptin levels in the general control group (Group 1) which was not exposed to any procedure were higher than those in Groups 2, 3 and 4 and lower than those in Groups 5, 6, 7 and 8 (p<0.01, Figure 1). Plasma leptin levels in ovariectomy control group (Group 3) were higher than those in Groups 2 and 4 and significantly lower than those in all other groups (p<0.01, Figure 1). Plasma leptin levels in control exercise group (Group 2) and ovariectomy exercise group (Group 4) were not different; but the levels in these two groups were found significantly lower than those in other groups (p<0.01, Figure 1).

Plasma lactate levels of the study groups were presented in Table 1. The comparison of plasma lactate levels among groups showed that the levels in Groups 2 (control exercise) and 4 (ovariectomy exercise) were significantly higher than those in all other groups (p<0.01, Table 1). Plasma lactate levels in these two groups were not different from each other. Plasma lactate levels were significantly lower in Groups 1 (general control), 3 (ovariectomy control), 5 (boron control) and 8 (ovariectomy+boron supplemented) than in other groups (p<0.01), but the levels in the mentioned groups (Groups 1, 3, 5, 8) were not different from each other (Table 1). Plasma lactate levels in Groups 6 (boron exercise) and 7 (ovariectomy boron exercise) were significantly lower than those in Groups 2 (control exercise) and 4 (ovariectomy exercise) (p<0.01, Table 1) and higher than those in Groups 1, 3, 5 and 8 (p<0.01, Table 1). There was no significant difference between plasma lactate levels of Groups 6 and 7.

DISCUSSION
Results of the studies investigating possible relationship between female reproduction system and leptin in rats have been controversial. While it is claimed that female reproductive hormones have an important role in regulating leptin levels (Shimizu et al. 1997; Ozcelik et al. 2004), there are also studies reporting that estrogen does not have a significant effect on leptin production (Watanobe et al. 1999). It can be said that there is no agreement between the results of studies on the re-
lation between ovariectomy and leptin in rats. Pinilla et al. (1999) claimed that ovariectomy increased leptin levels in rats, while Shimizu et al. (1997) showed that leptin secretion was inhibited in ovariectomized rats. The present results demonstrate that ovariectomy suppresses leptin secretion in rats. In this respect, our findings are consistent with those of Shimizu et al. (1997). In the study that claimed that ovariectomy increased leptin secretion (Pinilla et al. 1999), estrogen was administered to rats after ovariectomy caused an increase in leptin levels. However, in the present study, we did not make hormone replacement in rats after ovariectomy. The contradiction between our findings and those of Pinilla et al. (1999) probably results from this difference in the experimental procedure.

Our findings demonstrate that an acute exercise significantly inhibits plasma leptin levels in rats. Although the results are contradictory, it is generally acknowledged that physical activity leads to changes in leptin secretion. Contrary to the findings claiming that exercise increased leptin concentration (Unal et al. 2005; Van-Aggel-Leijssen et al. 1999), Hickey and Calsbeek (2001) reported that exercise did not alter leptin levels. Similarly, Hilton and Loucks (2000) also concluded that exercise did not inhibit leptin production. Another study noted a decrease in leptin levels 9 hours after acute exercise (Nindl et al. 2002). The reports of the above-mentioned researchers appear not to support our findings. However, our findings are consistent with the results of the researchers (Karamouzis et al. 2002; Zaccaria et al. 2002), who reported that there were significant decreases in leptin levels after intensive exercise in long-distance swimmers and marathon runners. Pagan et al. (1999) showed that leptin levels decreased by about 30% in rats following a 30-minute acute swimming exercise and this study can be said to be the most important study supporting our results. That is because we also investigated the effects of a 30-minute acute exercise on leptin levels. The study is fairly important since we can make a one-to-one comparison between this study and ours.

We obtained the highest plasma leptin levels in boron control, boron + exercise and Ovx-boron-exercise. Plasma leptin levels in these three groups were higher than those in all others. However, the levels in these three groups were not different from each other. We could not find a study in the literature investigating the relation between boron and leptin. However, our findings demonstrate that boron administration prevented the inhibition in leptin levels brought about by exercise or ovariectomy. It is stated that dietary boron supplement in athletes increases endogenic steroid production, is beneficial for performance and that side effects can be observed only when taken in high doses (Ahrrendt, 2001). It is reported that boron mineral is an additional element that can provide muscle enhancement to athletes during endurance exercise. This claim is based on the study that showed that 3 mg of boron supplement increased estradiol and testosterone levels (Sheng et al. 2001). However, subsequent studies reported that 2.5 mg/day boron supplement for 7 weeks did not have any ergogenic value in testosterone levels, body composition and resistance during endurance exercise (Kreider, 1999). Studies conducted on athletes verified that boron supplement increased serum testosterone, but showed that it did not affect muscle development (Ferrando and Green, 1993).

It is known that leptin is plays an important role in the regulation of energy balance (Hickey & Calsbeek, 2001). It is inevitable that leptin, which is an important hormone for the energy balance, is related with exercise and effective on performance (Hickey & Calsbeek, 2001). In the study hereby, boron administration resulted in an increase in leptin levels. Boron administration at least prevents the reduction in leptin levels caused by both exercise and ovariectomy. However, further studies are needed to explain the mechanisms playing a role in boron-exercise, boron-leptin and boron-female reproductive system relations.

The highest plasma lactate levels in the present study were found in control exercise and Ovx-exercise rats. Plasma lactate levels in boron-exercise and Ovx-boron-exercise were significantly lower than those in Groups 2 and 4. These findings of ours suggest that boron administration in exercise leads to inhibition of lactate levels. However, lack of any relevant study investigating the relationship between boron administration and lactate makes it difficult to discuss the findings we obtained. In the study carried out by Baltaci et al. (2003), a negative relation was reported between plasma lactate levels and leptin in rats which were made to do acute swimming exercise. In other words, as leptin levels increase, lactate levels decrease and vice versa. In the present study we also found that leptin levels in the groups that received boron were higher than those in other groups. Parallel to this, we found reduced lactate levels in groups with high leptin levels. However, the point that needs to be addressed here is whether boron administration affects lactate by increasing leptin levels. The findings put forth in this study demonstrate that boron administration increased leptin levels and inhibited lactate levels. Studies about the relation between boron and exercise concentrate generally on the relation of this element to testosterone hormone, muscle development and bone metabolism (Beattie & Peace, 1993; Naghi, 1999; Sheng et al. 2001). Even this aspect of the studies’ results suggest that boron administration could have positive effects on physical performance. Likewise we aimed to investigate primarily the effects of boron administration on exercise in ovariectomized rats. Recent studies make it evident that administration of estrogen is not sufficient in menopause treatment, and that several minerals including boron should be administered as treatment (Nielsen et al. 1987).

Various studies investigating the interaction between boron and reproductive system show that boron
administration contributes to such significant processes as endocrine system, calcium and bone metabolism, and bone development. The present findings are likely to make an additional contribution to what is known on this topic.

CONCLUSION

Depending on the findings obtained in this study it can be said that

1. Ovariectomy in rats leads to an inhibition of leptin secretion;
2. Acute swimming exercise significantly inhibits plasma leptin;
3. Boron administration prevents the inhibition of leptin levels in both ovariectomized rats and acute swimming exercise; and
4. Boron administration prevented the increase in circulating lactate concentrations induced by swimming exercise.

Parallel to the information above, it can be concluded that physiologic doses of boron administration can be beneficial for performance.

REFERENCES